OL-059 Penicillin-binding protein 2B gene (pbp2b) based PCR and sequencing for screening *Streptococcus pneumoniae* infection and predicting its susceptibility in cerebral spinal fluid from paediatric bacterial meningitis patients

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Background: To evaluate the penicillin-binding protein 2B gene (*pbp2b*) based PCR and sequencing for screening *Streptococcus pneumoniae* infection and predict its susceptibility in cerebral spinal fluid from paediatric bacterial meningitis patients.
Methods: A nested PCR targeting *pbp2b* and another two *S. pneumoniae* specific PCR targeting pneumolysin (*ply*) and autolysin (*lytA*) were developed for detection of *S. pneumoniae* in cerebral spinal fluid from bacterial meningitis patients. The PCR results of three different genes and culture were compared. The consistency of penicillin susceptibility PCR (using resistant and susceptible primers respectively), sequencing and culture based phenotypic penicillin resistant results were compared.
Result: Of the 161 specimens studied, there were totally 25 *S. pneumoniae* infection confirmed by different methods (16 by *lytA* PCR, 14 by *ply* PCR, 16 by *pbp2b* PCR and 9 by cerebrospinal fluid culture). Of the 16 *pbp2b* positive specimens, penicillin sensitive and resistant sequence types account for half respectively. 4 of the 16 *pbp2b* positive specimens had culture based phenotypic penicillin-resistant result. 3 of 4 were consistent with penicillin susceptibility PCR result. The result of susceptibility PCR targeting *pbp2b* was consistent with sequencing result. There were no new point mutations but new sequence types were found in these strains when compared with GenBank. Penicillin resistant in pneumococcal meningitis was 66.67% (6/9) by culture phenotype and 50% (8/16) by PCR and sequencing when culture was negative.
Conclusion: *pbp2b* can be served as a good target gene to detect *S. pneumoniae* and predict its penicillin susceptibility, especially important when culture was negative.

OL-060 In vitro activity of antibiotics on biofilm producing isolates of *Pseudomonas aeruginosa*

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Objectives: *Pseudomonas aeruginosa* is a known biofilm producer especially in the lung of patients suffering from Cystic fibrosis. We undertook this study to check for biofilm producing capability of *Pseudomonas aeruginosa* isolated from various clinical specimens and also to check their suspectibility pattern against anti-pseudomonal antibiotics in single and in various combinations.
Methods: The present study was conducted in the Department of Microbiology on 100 consecutive clinically significant isolates of *Pseudomonas aeruginosa*. Isolates were identified and their antibiogram was performed following standard methods. All these isolates were tested for biofilm production following the method described by Stepanovic *et al*. Minimum inhibitory concentration against Ceftazidime, Ciprofloxacin, Rifampicin and Colistin was determined by Agar dilution method. A total of ten biofilm producing isolates were randomly selected to study the effect of various combinations of antibiotics at various concentrations by Microbroth dilution method.
Results: Out of hundred consecutive isolates of *Pseudomonas aeruginosa* isolated from clinical samples, 78% were positive for biofilm production. Out of these, 41.66% were strong biofilm producers, 25% were moderate and 11.66% were weak producers. Colistin and Rifampicin demonstrated synergy in 9/10 isolates whereas Ceftazidime and Ciprofloxacin showed synergy only in 3/10 strains tested.
Conclusion: Biofilm formation is commonly associated with *Pseudomonas aeruginosa* isolated from various clinical samples. Colistin and Rifampicin was the most effective combination followed by Colistin and Ceftazidime against biofilm producing isolates.

OL-061 Genotypic characterization of *Vibrio cholerae* isolated in Malaysia based on multilocus sequencing typing (MLST) and multi-virulence locus sequencing typing (MVLST)

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Background: In Malaysia, cholera outbreaks due to the *V. cholerae* O1 serotype occur periodically. Non-O1/non-O139 *V. cholerae* is also frequently isolated from seafood and water sources but has not been implicated in any major outbreaks. A variety of genotyping methods have been developed for this pathogen by assessing the variations of genome composition.
Methods: In the present study, multilocus sequencing typing (MLST) and multi-virulence locus sequencing typing (MVLST) assays were used to determine the genetic variation and relatedness of 43 *V. cholerae* strains of different serogroups isolated from various sources in Malaysia. The MLST assay comprised of 6 housekeeping genes (*dnaE*, *lap*, *recA*, *gyrB*, *cat* and *gmd*) while the MVLST assay incorporated 3 virulence genes (*ctxAB*, *tcpA* and *tcpI*) and 3 virulence-associated genes (*hlyA*, *toxR* and *rtxA*).
Results: For MLST analysis, *dna*E and *rtx*A were the most conserved genes in *V. cholerae* O1 strains. Of the 43 strains, 29 sequence types were observed and this assay was capable to differentiate within the *V. cholerae* non-O1/non-O139 strains. On another hand, 5 *V. cholerae* O1were found to be the El Tor variant O1 strains as they harboured the classical *ctx*B. In addition, the classical *ctx*B was also observed in O139 *V. cholerae*. A total of 27 sequence types were obtained from MVLST analysis. Moreover, MVLST was able to distinguish the outbreak strains from 2 localities, differentiate the O1 *V. cholerae* based on the source of isolation, and discriminate the toxigenic strains from the non-toxigenic strains.
Conclusion: The 43 *V. cholerae* strains were characterized based on their serogroups and toxigenicity. The MLST and MVLST analyses in this study are useful and suitable for the local epidemiology study of *V. cholerae*.

OL-062 Adherence comparison and antiserum titer determination of three major outer membrane protein TSA56, TSA47 and TSA22 in *Orientia tsutsugamushi*

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Background: *Orientia tsutsugamushi* (OT) is a gram-negative obligate intracellular bacterium in the family Rickettsiaceae and is the causative organism of scrub